REMARKS/ARGUMENTS

I. Status of the Claims

Claims 1, 3, 5, 6, and 7 will be pending after entry of this amendment. Claim 1 has been amended to more clearly define the invention. Support for the amendment can be found in the specification, for example, on page 5, lines 8-10. The amendment is necessary and was not earlier presented because it is in response to new grounds of rejection set forth in the final Office Action. Since the amendment obviates the outstanding grounds of rejection as discussed below, reduces the number of issues, contains no new matter, and places the application in condition for allowance or better condition for appeal, the amendment should be entered.

II. The Claims Are Patentable Over the Cited References

Claims 1, 3, 5, and 6 have been rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Gallimore *et al.* (*J. Exp. Med.* **187**: 1383-1393, 1998). Applicants traverse the rejection.

To establish a *prima facie* case of obviousness, there must be some suggestion or motivation to modify the reference or to combine the reference teachings so as to arrive at the claimed invention and there must be a reasonable expectation of success for achieving the claimed invention as a whole. *See In re Vaeck*, 20 U.S.P.Q. 2d 1438 (Fed. Cir. 1991). For the reasons discussed below, a proper *prima facie* case of obviousness has not been set forth.

Independent claim 1 is now directed to a method for detection of antigen specific T cells comprising (a) providing a recombinant cell expressing an MHC class I protein-fluorescent protein fusion molecule or a radiolabeled MHC class I protein on a surface of the recombinant cell; (b) contacting the MHC class I protein-fluorescent protein fusion molecule or the radiolabeled MHC class I protein, bound to a specific antigen with a population of T cells; (c) incubating the fusion molecule or the radiolabeled MHC class I protein, bound to the specific antigen together with the population of T cells for a period of time sufficient for the T cells to internalize the fusion molecule or the radiolabeled MHC class I protein from the T cell surface; and (d) identifying the T cells that have internalized

the fusion molecule or the radiolabeled MHC class I protein. The cited reference fails to teach or suggest such a method.

The Gallimore *et al.* reference fails to teach or suggest a method as defined in any of claims 1, 3, 5, and 6 in which the method provides, in part, a recombinant cell expressing an MHC class I protein-fluorescent protein fusion molecule on a surface of the recombinant cell.

The Office Action cites the Gallimore *et al.* reference as teaching a method for the purification of antigen-specific T cells. The Examiner argued that the Gallimore *et al.* reference utilizes "MHC class I protein bound to an antigen further complexed with a phycoerythrin (PE) label, followed by fluorescence activated cell sorting, ... [and] that a variety of detectable markers, as well as complexes comprising partners might be employed in the claimed method." However, in contrast to the presently claimed method, the Gallimore *et al.* reference utilized *purified* MHC class I-peptide complexes. See, for example, Gallimore *et al.*, page 1385, column 1, paragraph 2. The presently claimed method employs a recombinant cell expressing an MHC class I protein-fluorescent protein fusion molecule on a surface of the recombinant cell. Therefore, the recombinant cell expressing the fusion molecule on the cell surface can act as an antigen presenting cell (APC), and the MHC class I protein-fluorescent protein fusion molecule can be acquired and *internalized* by the T cell. Furthermore, the Gallimore *et al.* reference does not teach or suggest the step of identifying the T cells that have internalized the fusion molecule by detecting fluorescent emission of the fluorescent protein fusion molecule in a fluorescence activated cell sorter because the Gallimore *et al.* reference utilizes purified complexes which are not internalized by the T cell. By contrast, the present claimed invention provides, in part, a recombinant cell expressing an MHC class I protein-fluorescent protein fusion molecule on a cell surface and presents the fusion protein which is internalized by the T cell. From a proper reading of the Gallimore *et al.* reference there can be no reasonable argument that it teaches or even suggests the presently claimed methods. Since the claims patentably define over the prior art, Applicants respectfully request that the rejection of claims 1, 3, 5, and 6 under 35 U.S.C. § 103(a) be withdrawn.

Claim 1 has been rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by U.S. patent 5,284,935 ("the '935 patent"). Applicants traverse the rejection.

The '935 patent fails to teach or suggest a method as defined in claim 1, in which a method for detection of antigen specific T cells comprises, in part, providing a recombinant cell expressing an MHC class I protein-fluorescent protein fusion molecule or a radiolabeled MHC class I protein on a surface of the recombinant cell. The Examiner stated that the '935 patent teaches "a method for the detection of antigen specific T cells comprising contacting, incubating and identifying T cells with a fusion protein comprising a MHC Class I, a peptide, and a fluorescent label." The '935 patent teaches, however, complexes of MHC, peptide, and label that are purified. The '935 patent defines an "isolated MHC component" as an MHC glycoprotein "in other than its native state, for example, not associated with the cell membrane of a cell that normally expresses MHC." See, for example, '935 patent, column 6, lines 35 to 42. The '935 patent does not disclose or suggest a method for detection of antigen specific T cells as defined in claim 1 which comprises, in part, providing a recombinant cell expressing an MHC class I protein-fluorescent protein fusion molecule or a radiolabeled MHC class I protein on a surface of the recombinant cell. Since the claims patentably define over the '935 patent, Applicants respectfully request that the anticipation rejection of claim 1 under 35 U.S.C. § 102(b) be withdrawn.

Claims 3, 5, and 6 have been rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the '935 patent. Applicants also traverse the rejection.

Claims 3, 5 and 6 are directed to a method for detection of antigen specific T cells comprising, in part, providing a recombinant cell expressing an MHC class I protein-fluorescent protein fusion molecule on a surface of the recombinant cell, and identifying the T cells that have internalized the fusion molecule by detecting fluorescent emission of the fluorescent protein fusion molecule in a fluorescence activated cell sorter. The '935 patent fails to teach or suggest a method. As explained above, the '935 patent teaches a purified complex of MHC, peptide, and label. Moreover, the '935 patent does not teach or suggest a recombinant cell expressing an MHC class I protein-fluorescent protein fusion molecule on a cell surface. Since the claims patentably define over the prior art, Applicants respectfully request that the rejection of claims 1, 3, 5, and 6 under 35 U.S.C. § 103(a) be withdrawn.

Claim 7 has been rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Gallimore *et al.* (*J. Exp. Med.* **187**: 1383-1393, 1998) or over U.S. patent 5,284,935 ("the '935 patent") in view of Cai *et al.* (*Proc. Natl. Acad. Sci. USA* **93:** 14736-14741, 1996). Applicants traverse the rejection.

Claim 7 is directed to a method for detection of antigen specific T cells comprising, in part, providing a recombinant cell expressing an MHC class I protein-fluorescent protein fusion molecule or a radiolabeled MHC class I protein on a surface of the recombinant cell, wherein the recombinant cell is a *Drosophila* cell, and identifying the T cells that have internalized the fusion molecule or the radiolabeled MHC class I protein. The Gallimore *et al.* reference or the '935 patent in view of the Cai *et al.* reference fails to teach or suggest the claimed method.

The Office Action argues that the method of the Gallimore *et al.* reference and the '935 patent differ from the claimed method only in that they do not employ a recombinant *Drosophila* cell, and that the Cai *et al.* reference teaches the benefits of using a recombinant *Drosophila* cell as a platform for testing APC function. As noted above, the Gallimore *et al.* reference and the '935 patent teach a *purified* complex of MHC, peptide, and label. The Cai *et al.* reference fails to cure the deficiencies of the Gallimore *et al.* reference and the '935 patent. The Cai *et al.* reference discloses a recombinant *Drosophila* cell. However, the combination of references do not teach or suggest the claimed method for detection of antigen specific T cells comprising, in part, identifying the T cells that have internalized the fusion molecule or the radiolabeled MHC class I protein. Applicants' claimed invention demonstrates that an MHC peptide complex can be acquired *and internalized* by a T cell, and thus *identifying* the T cells that have internalized the fusion molecule or the radiolabeled MHC class I protein. From a proper reading of the Gallimore *et al.* reference or the '935 patent in view of the Cai *et al.* reference, there can be no reasonable argument that it teaches or even suggests the presently claimed methods. Since the claims patentably define over the prior art, Applicants respectfully request that the rejection of claim 7 under 35 U.S.C. § 103(a) be withdrawn.

In making the various obviousness rejections based on the above-cited prior art, the Examiner has not addressed how the cited references would have suggested the claimed

invention as a whole. An analysis of obviousness of a claimed combination must include consideration of the results achieved by it. Resulting advantages of the claimed invention are reflected in the enclosed Huang *et al.* article ("TCR-Mediated Internalization of Peptide-MHC Complexes Acquired by T Cells," *Science*, **286:** 952-954, 1999) attached as Exhibit 1. Focusing on the obviousness of substitutions and differences as the Examiner has done here, instead of the invention as a whole, is a legally improper way to simplify the determination of obviousness. *Gillette Co. v. S.C. Johnson & Son, Inc.*, 919 F.2d 720, 16 USPQ2d 1923 (Fed. Cir. 1990).

III. Conclusion

In view of the foregoing, the application is now in condition for allowance. The prompt issuance of a formal Notice of Allowance is therefore requested.

If the Examiner believes a telephone conference would expedite allowance of this application, please telephone the undersigned at 206-332-1380.

Date: June 16, 2004

Phillip A. Singer
Registration No. 40,176

Enclosure: Exhibit 1: "TCR-Mediated Internalization of Peptide-MHC Complexes Acquired by T Cells," *Science*, **286:** 952-954, 1999

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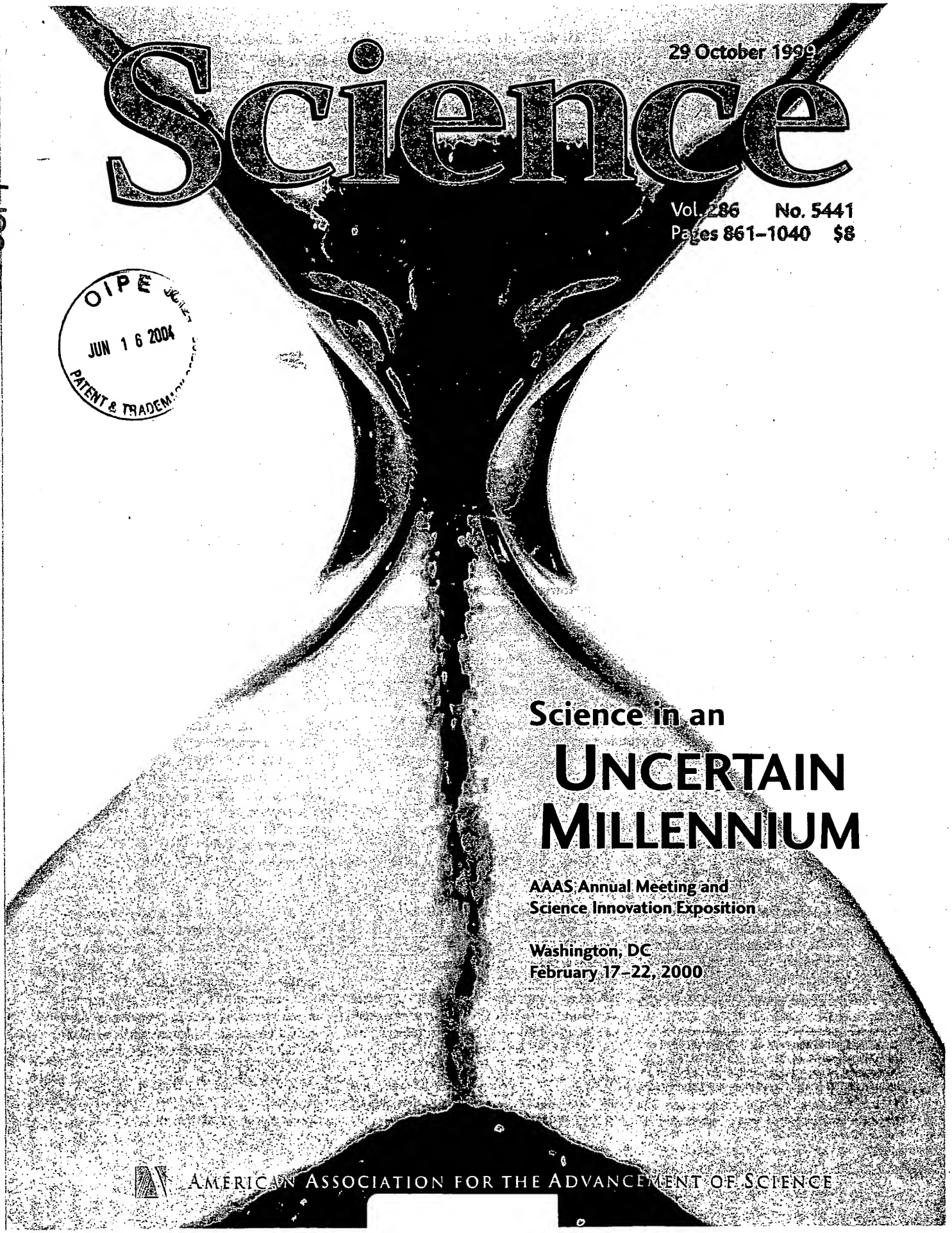

Science in an UNCERTAIN MILLENNIUM

AAAS Annual Meeting and Science Innovation Exposition

**Washington, DC
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EXHIBIT 1

COVER The 2000 AAAS Annual Meeting and Science Innovation Exposition will convene on 17 through 22 February in Washington, DC, with symposia, exhibits, and lectures examining the exciting diversity of science, technology, and engineering. The vital role of science and technology in the 21st century is captured by the meeting's theme, "Science in an Uncertain Millennium." For expanded program details and advance registration rates, see pages 980 to 1001.

TCR-Mediated Internalization of Peptide-MHC Complexes Acquired by T Cells

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Peptide–major histocompatibility complex protein complexes (pMHCs) on antigen-presenting cells (APCs) are central to T cell activation. Within minutes of peptide-specific T cells interacting with APCs, pMHCs on APCs formed clusters at the site of T cell contact. Thereafter, these clusters were acquired by T cells and internalized through T cell receptor–mediated endocytosis. During this process, T cells became sensitive to peptide-specific lysis by neighboring T cells (fratricide). This form of immunoregulation could explain the "exhaustion" of T cell responses that is induced by high viral loads and may serve to down-regulate immune responses.

T cell responses are initiated by T cell receptor (TCR) recognition of pMHCs on APCs (*1*). Upon specific interaction of T cells with APCs, TCR and MHC molecules are assembled at the center of supramolecular activation clusters (*2*). The fate of these TCR-MHC clusters at the T cell–APC contact site is unclear. However, it is known that interaction of TCRs with pMHC complexes is followed by TCR down-regulation (*3*) and that T cell–APC interaction can cause APC-derived surface molecules to adhere to the surface of T cells (*4*).

To investigate the fate of MHC clusters at the T cell–APC contact sites, we used *Drosophila*, RMA-S, and dendritic cell lines expressing MHC class I (L^d)–green fluorescent protein fusion molecules (L^d-GFP) (*5*) as APCs to activate CD8 T cells from the 2C TCR transgenic mouse line (*6*, *7*). 2C T cells recognize L^d plus either QL9 peptide or a closely related peptide, p2Ca (*8*). Empty L^d-GFP molecules expressed on the surface of RMA-S and *Drosophila* cells can be efficiently loaded with exogenous peptides (*7*, *9*). Within 5 min of 2C T cells interacting with either *Drosophila* cells (Fly.L^d-GFP) or RMA-S cells (RMA-S.L^d-GFP) plus the QL9 peptide, L^d-GFP molecules formed large clusters at the T cell–APC contact site [Fig. 1A and Web figure 1 (*10*)]. After 30 min, the L^d-GFP clusters at the T cell–APC interface decreased in size, and small aggregates of L^d-GFP appeared concomitantly within 2C T cells at sites distal to the contact site [Fig. 1, B and C, Web figure 2 (*10*)]. This process was not seen with P1A (*11*) (Fig. 1D), a control peptide that binds to L^d but is not recognized by 2C TCR (*7*). Aggregates of L^d-GFP were also detected inside 2C T cells after interaction with the L^d-GFP–transfected dendritic cell line DC2.4 (*12*) (Fig. 1E), which presents endogenous p2Ca peptide. To exclude the possibility that GFP itself could mediate the acquisition of L^d by T cells, we used *Drosophila* cells expressing untagged L^d (no GFP) loaded with QL9 peptide as APCs. After culture for 1 hour, multiple aggregates of L^d molecules were detected in 2C T cells by intracellular staining with monoclonal antibody (mAb) to L^d (*13*) (Fig. 1F). Thus, in the presence of antigenic peptides, L^d molecules can be acquired from APCs by T cells, and this acquisition is independent of GFP.

Acquisition of APC-derived L^d molecules by 2C T cells was further demonstrated by immunoprecipitation (Fig. 1, G and H). After culture with ^{35}S-methionine–labeled fibroblasts (L cells) transfected with L^d (L-L^d) (*14*), 2C T cells were highly purified and immunoprecipitation was performed with mAbs to class I molecules. Immunoprecipitation of L^d from 2C T cells cultured with L-L^d plus QL9 peptide was prominent (Fig. 1G). In the presence of the control P1A peptide (*11*), precipitation of L^d was limited, but clearly detectable, presumably due to the presentation of endogenous p2Ca peptide in L-L^d cells. In contrast, other MHC class I molecules (K^k and D^k) expressed by L cells were not detectable in 2C T cells by immunoprecipitation (Fig. 1G). The peptide-

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dependent acquisition of L^d molecules by T cells could be blocked by adding mAbs either to TCR or L^d during the culture (Fig. 1H). Thus, acquisition of L^d by T cells requires a specific interaction between TCRs and pMHC complexes.

The kinetics of acquisition of APC-derived pMHC molecules by T cells was investigated by fluorescent-activated cell sorting (FACS) analysis. After culture with Fly.L^d-GFP plus peptides for 30 min, most 2C T cells were positive for L^d-GFP with QL9 peptide but negative with the control P1A peptide (Fig. 2A). The amount of L^d-GFP on 2C T cells remained high for 2 hours, then gradually declined over 6 hours (Fig. 2B). Peptide-titration studies showed that the amount of L^d-GFP acquired by 2C T cells correlated with the concentration of QL9 peptide used in the culture (Fig. 2C). With p2Ca, which is a lower affinity peptide than QL9, acquisition of L^d-GFP was substantial but less marked than with QL9. Similar results were obtained by FACS analysis of 2C T cells stained with mAb to L^d (*13*) after culture with *Drosophila* cells expressing untagged L^d (Fly.L^d) (Fig. 2, B and C). Thus, acquisition of L^d molecules by 2C T cells is dependent on time and specific peptide concentration.

Rapid acquisition of L^d molecules by T cells correlated with down-regulation of 2C TCRs (Fig. 2). Because TCR down-regulation is a result of TCR internalization by T cells (*15*), 2C TCRs might be cointernalized with acquired L^d. In support of this possibility, L^d-GFP colocalized with TCRs in 2C T cells that were cultured with Fly.L^d-GFP plus QL9 peptide but not P1A peptide (Fig. 3A). L^d-GFP internalized by 2C T cells partly colocalized with transferrin-containing vesicles (Fig. 3B). To follow the intracellular fate of L^d-GFP, we used LysoTracker, a red fluorescent dye that specifically accumulates in low-pH compartments of cells, such as endosomes and lysosomes (*16*). L^d-GFP appeared in acidic compartments of 2C T cells cultured with APCs for 3 hours (Fig. 3C). Thus, MHC molecules acquired by T cells were cointernalized with TCRs through endocytosis and localized in endosomes and lysosomes.

The presence of APC-derived pMHC complexes on the T cell surface, detected by FACS analysis (Fig. 2), could make these T cells susceptible to lysis by neighboring T cells with peptide-specific cytotoxic T lymphocyte (CTL) activity (fratricide). To test this possibility, we first sensitized ^{51}Cr-labeled activated T cells with APCs expressing pMHC and then added fresh CTLs to the culture to perform a standard ^{51}Cr-release assay. Pre-culturing ^{51}Cr-labeled 2C T cells ($H2^b$, L^d) with L^d-expressing APCs loaded with QL9 peptide (but not P1A peptide) rendered the labeled 2C T cells susceptible to lysis by unlabeled 2C CTLs (Fig. 4, A and B). The lysis of ^{51}Cr-labeled 2C T cells was blocked by mAbs to 2C TCR (1B2) (Fig. 4A) or to L^d (Fig. 4B). To rule out the possibility that peptides released from the killed APCs could sensitize the 2C T cells for subsequent killing, we used RMA-S cells (K^bD^b) loaded with a mixture of SIY peptide (*8*) and GP33 peptide (*17*) to sensitize either 2C T cells or LCMV T cells (*17*); 2C T cells recognize K^b-SIY but not D^b-GP33, whereas LCMV T cells recognize D^b-GP33 but not K^b-SIY (*17*). The ^{51}Cr-labeled 2C T cells that were sensitized with RMA-S cells loaded with both peptides were lysed only by 2C CTLs and not by LCMV CTLs (Fig. 4C). Conversely, ^{51}Cr-labeled LCMV T cells sensitized with RMA-S cells plus both peptides were killed only by LCMV CTLs and not by 2C CTLs (Fig. 4D). Thus, T cells were sensitized for fratricide by pMHC complexes specifically acquired from target cells. With ^{51}Cr-labeled 2C T cells, sensitizing the T cells for lysis required a high concentration of QL9 peptide on L^d-expressing APCs

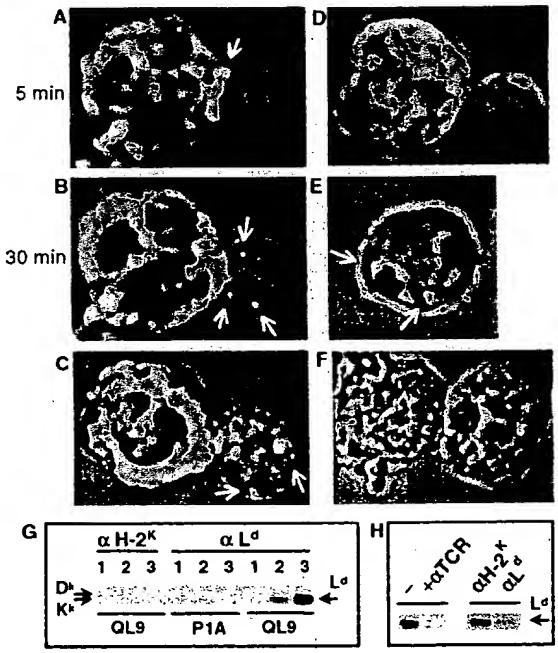

Fig. 1. Acquisition of APC-derived MHC class I molecules by T cells. (A to E) CD8 2C T cells were cultured with APCs expressing L^d-GFP. GFP fluorescence and differential interference contrast (DIC) images obtained with a confocal microscope system (FluoView, Olympus) were overlaid. (A and B) GFP fluorescence and DIC images of a pair of resting 2C T cell-Fly.L^d-GFP loaded with QL9 peptide were acquired every 30 s for 45 min. Images at 5 and 30 min are shown. (C) Image of one pair of activated 2C T cell-RMA-S.L^d-GFP with QL9 peptide after 30 min of culture. (D) One pair of resting 2C T cell-Fly.L^d-GFP with P1A peptide. (E) Image of an activated 2C T cell after culture for 45 min with the L^d-GFP–transfected DC line. (F) Immunofluorescence image of one pair of activated 2C T cell-Fly.L^d. Activated 2C T cells were cultured for 1 hour with *Drosophila* cells expressing untagged L^d in the presence of QL9 peptide. Cells were fixed, permeabilized, and stained with biotinylated mAb to L^d, followed with strepavidin–Texas Red. (G) Peptide-specific immunoprecipitation of APC-derived L^d from resting 2C T cells. 2C T cells (lanes 1, 2, and 3: no T cells, 2×10^7 T cells, and 4×10^7 T cells, respectively) were cultured for 4 hours with 3×10^6 ^{35}S-methionine–labeled L-L^d cells. Lysates were prepared and immunoprecipitated with mAbs to $H2^K$ or to L^d. (H) Immunoprecipitation of L^d molecules from 2C T cells cultured with L-L^d cells plus QL9 peptide for 4 hours with or without mAbs to 2C TCR (αTCR), to L^d (αL^d), or to H-2^K (α$H2^K$).

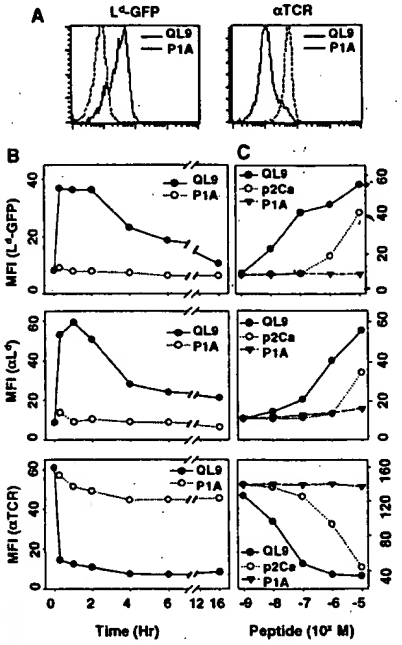

Fig. 2. Time- and peptide dose–dependent acquisition of APC-derived MHC molecules and TCR down-regulation. Resting 2C T cells were cultured for the indicated time with Fly.L^d -GFP APCs plus the indicated doses of peptides. The amount of L^d-GFP acquired by CD8 2C T cells was directly analyzed by FACS. For the surface level of L^d and TCRs, 2C T cells were cultured with Fly.L^d APCs plus the indicated doses of peptides and analyzed with mAbs to L^d (αL^d) or to 2C TCR (αTCR) by FACS. (A) Histograms showing the amount of L^d-GFP and TCR on 2C T cells after culture for 30 min with *Drosophila* APCs plus 10 μM of QL9 or P1A peptide. (B) Kinetics of TCR down-regulation and acquisition of L^d by 2C T cells with 10 μM of the indicated peptides. (C) Peptide-dose dependent TCR down-regulation and acquisition of L^d by T cells.

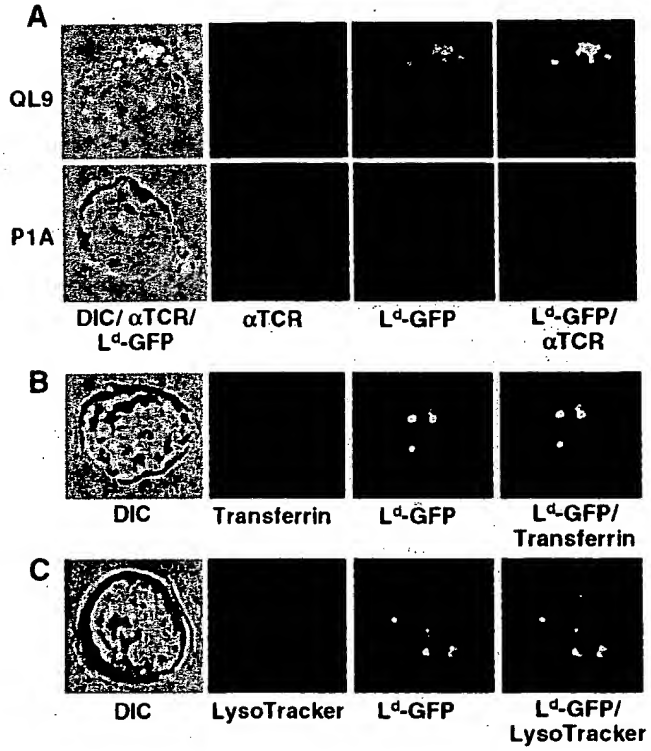

Fig. 3. TCR-mediated endocytosis of APC- derived MHC class I molecules. (A) Intracellular colocalization of TCR and L^d-GFP in 2C T cells. After culture for 1 hour with Fly.L^d-GFP APCs plus QL9 or P1A peptide, 2C T cells were permeabilized and stained first with a mixture of biotinylated mAbs to TCR (anti-CD3ε, anti-TCRβ, and a clonotypic mAb, 1B2) (αTCR) and then with streptavidin–Texas Red. (B) Activated 2C T cells were cultured first with Texas Red–conjugated transferrin (5 μg/ml) and then with Fly.L^d-GFP plus the QL9 peptide for 30 min. (C) Activated CD8 2C T cells were cultured with RMA-S.L^d-GFP plus QL9 for 3 hours and then stained with 5 nM LysoTracker Red DND-99 for 10 min.

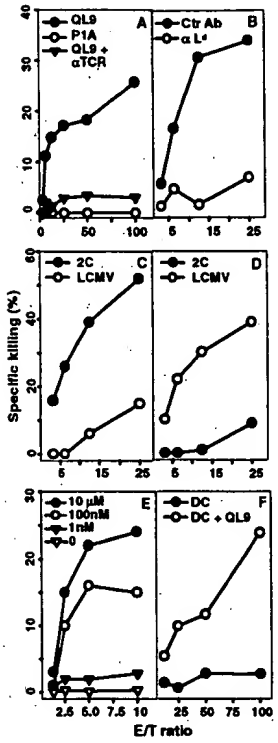

Fig. 4. Sensitization of CD8 T cells to peptide-specific lysis by CTLs. Activated T cells were labeled with ^{51}Cr and then sensitized with APCs for 1 hour. During the killing phase, fresh unlabeled CTLs were added to the culture. After 4 to 6 hours, ^{51}Cr specifically released from the sensitized T cells was measured. (A) ^{51}Cr-labeled 2C T cells were sensitized by Fly.L^d with QL9 or P1A peptide. During the killing phase with 2C CTL, anti-2C TCR mAb (αTCR) was added to the culture. (B) Labeled 2C T cells were sensitized by RMA-S.L^d plus QL9 peptide in the presence of anti- L^d mAb (αL^d), followed by culture with 2C CTLs. (C and D) Labeled 2C T cells were sensitized with RMA-S cells loaded with a mixture of SIY and GP33 peptides, and then either 2C CTLs or LCMV CTLs were used during the killing phase. (E) Labeled 2C T cells were sensitized with RMA-S.L^d cells loaded with the indicated doses of QL9 peptide, followed by culture with 2C CTLs. (F) Labeled 2C T cells were sensitized by purified DCs ($H2^d$) from mouse spleen with or without added QL9 peptide (10 μM), followed by culture with 2C CTLs.

(Fig. 4). Likewise, with dendritic cells ($H2^d$) as APCs, sensitizing 2C T cells required that the DC (which expresses endogenous p2Ca peptide) be supplemented with a high concentration of QL9 peptide.

Fratricide of CD8 T cells after antigen-specific T cell–APC interaction could be relevant to the finding that in vivo exposure to high doses of virus causes antigen-specific CD8 T cells to proliferate briefly and then disappear (*17*). The mechanism of such CTL "exhaustion" is not clear. It has been suggested that the responding T cells themselves are infected with the viruses, thus becoming targets for CTLs (*18*). Nevertheless, detection of viruses in CTLs is not an invariable finding (*17*). Our data (Fig. 4) suggest that fratricide of T cells does not require direct viral infection of T cells but can be accomplished through transfer of preformed pMHC complexes from the APC to specific T cells. On the basis of the peptide-dose experiment (Fig. 4E), T cell fratricide would only occur if a high density of pMHC was transferred to the surface of the target T cells, which could happen in cells infected by high doses of virus. Because unrestricted T cell responses upon exposure to high doses of viruses would lead to dangerous immunopathology (*19*), reducing the intensity of the immune responses by fratricide of T cells could be a beneficial form of immunoregulation in vivo.

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